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Antioxidant vitamins and cancer risk: is oxidative damage to DNA a relevant biomarker?

Abstract Oxidative damage to DNA is regarded as an important step in carcinogenesis. These lesions may arise as a consequence of exposure to xenobiotics, but are also generated as a consequence of endogenous generation of oxidizing compounds. Measurements of oxidative damage to guanines, such as 8-oxo-7, 8-dihydroguanine (8-oxodG) are increasingly being regarded as reliable biomarkers of oxidative stress and they may have a predictive value of cancer risk, although this needs to be established independently in several cohort studies. A survey of intervention studies of the ingestion of antioxidant-containing foods or tablets of antioxidants indicate that about one-third of the studies reported a protective effect in terms of lower levels of oxidative damage to DNA in white blood cells or decreased urinary excretion of 8-oxodG. Although firm conclusions cannot be reached, there appears to be links between ingestion of antioxidants, oxidative damage to DNA, and risk of cancer.

Key words antioxidants – comet assay – 8-oxodG – oxidative stress – oxidative damage to DNA

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A role of oxidative damage to DNA in cancer development

Cancer is commonly regarded as a disease of the genes. An accumulation of somatic mutations, which is an important event of cancer development, has been documented in aged cells and tissues. This accumulation of mutations presumably relates to cumulative lifetime exposure to endogenous and exogenous DNA damaging agents. Food and beverages frequently contain carcinogens, partly those generated in processing, such as the cooking of meat, natural compounds and maybe some chemicals that are added to dietary products. In addition, it is possible that reactive oxygen species (ROS) are among the most important human carcinogens. ROS, which can be produced by various biochemical reactions, and as a byproduct of oxygen metabolism in mitochondria, can damage different types of cellular molecules such as proteins, lipids and nucleic acids.

Hydroxyl radicals are well characterized type of ROS that are found in biological materials. These

radicals cause the formation of a large number of pyrimidine- and purine-derived lesions in DNA [15], and some of these modified DNA bases have mutagenic potential [6, 32]. 8-Oxo-7, 8-dihydroguanine (8-oxoGua; and its deoxyribonucleoside equivalent, 8-oxodG) is one of the most widely studied lesions. In this context it is noteworthy that it has been estimated that each cell of the human body experiences about 2,000 oxidative modifications of guanine per day, based on the measurement of urinary excretion of 8-oxoGua and 8-oxodG that has been shown to be 2.5 nmol per kg per day [52]. In experimental systems, 8-oxoGua residues in DNA can lead to GC → TA transversions, unless repaired prior to DNA replication [8]. Therefore, the presence of 8-oxoGua in cells of whole animals and humans may lead to point mutations. However, in order to contribute significantly to the overall load of mutations in multi-cellular organisms, oxidative damage to DNA will need to occur at a sufficiently high frequency to exceed the capacity of the cell for DNA repair.

DNA mutation is considered to be a crucial step in carcinogenesis and elevated levels of oxidatively derived DNA lesions have been noted in many tumours, strongly implicating such damage in the aetiology of cancer [13]. Elevated levels of damage have been purported to arise as a result either of (1) the tumour having an environment low in antioxidant enzymes and high in ROS generation, or (2) there being reduced DNA repair [13]. Oxidative mechanisms have been demonstrated to possess a potential role in the initiation, promotion and malignant conversion (progression) stages of carcinogenesis. Since the cumulative cancer risk increases with the fourth power of age, and is associated with an accumulation of DNA damage, oxidative DNA damage has received increasing attention in relation to cancer. Lesions, such as 8-oxodG, are used as biomarkers of oxidative stress. Mutation is not the sole effect of oxidative damage to DNA, gene expression can be altered by damage to promoter sequences [19] or disturbance of DNA methylation patterns. Telomere shortening can be accelerated, and microsatellite instability increased (reviewed in [18]), all of which may be hallmarks of cancerous cells. This, together with the mutagenicity of such damage in mammalian cells, has lead to the proposal that they may be used as intermediate markers of cancer, although this concept has yet to be verified in prospective studies of cancer risk. At the same time, there are potentially serious problems in the measurement of 8-oxodG, connected with spurious oxidation of DNA during sample preparation. An extensive investigation by the European Standards Committee on Oxidative DNA damage (ESCODD) concluded that the true background level of 8-oxodG in mammalian cells is likely to be 0.3–4.2 lesions per 10^6 unaltered guanines and that reports outside this range should be viewed sceptically [9].

Elevated levels of ROS may lead to the activation of transcription factors, and their corresponding genes. This, together with increased levels of DNA damage, may create a selection pressure for the malignant phenotype seen in cancer [64]. Whilst such studies have furthered the hypothesis that oxidative damage to DNA may be an important risk factor for carcinogenesis, it has been argued that the mere presence of 8-oxodG in DNA is unlikely to be a necessary and sufficient cause of tumour formation. This is clearly illustrated by a large number of pathological conditions in which levels of oxidative damage to DNA are elevated, but with no increased incidence of carcinogenesis [13]. This raises some possibility that oxidative damage to DNA could be an epiphenomenon in relation to an on-going pathophysiological process, and elevated levels may not have a causal role in carcinogenesis. This hypothesis of reverse causality indicates that the mere presence of elevated levels of damage in tumours does not indicate that it was oxidative damage which led to the tumourigenic changes. Elevated levels of oxidized DNA in tumour tissue may have occurred as a result of well established characteristics of tumours, e.g. increased metabolism or cell turnover. In addition, for DNA mutations to arise from oxidative damage, the nuclei of undifferentiated, proliferating stem cells, have to be affected. In addition, the mutations should arise from oxidative damage to DNA within a coding region or other regulatory segments of the genome. Since tissue samples from both tumours and normal tissue represent a heterogeneous mixture of differentiated and undifferentiated cells (the former likely to predominate), the analytical procedures in current use are unable to quantify the level of lesions in the most important precursor cells. It is such issues that will have to be addressed before the link between oxidative damage to DNA and cancer can be firmly established. In this context there is a need for further in vitro, mechanistic investigations, as well as large prospective studies in order to demonstrate that a high level of oxidatively damaged DNA is related to an increased cancer risk. Ultimately, demonstrating that an intervention which reduces oxidative damage to DNA also reduces cancer risk, will provide the much needed evidence for the value of such biomarkers in maintenance of public health and cancer prevention.

Summing up, it can be argued from the data obtained so far that it appears likely that the development of many types of cancer involves oxidative stress, but that it is difficult, at present to determine to what extent oxidative stress is directly involved in carcinogenesis since full development of the disease,

following carcinogen exposure, may take 20–40 years. Inter-individual variation in response to carcinogens, such as xenobiotic metabolism and DNA repair, results in a highly heterogeneous population in which to study these effects, further complicating this issue. It is very difficult, therefore, to establish directly that the DNA lesion responsible for a carcinogenic process is the lesion present in tumours many generations of cells later. Nevertheless, one should bear in mind that DNA damage, altered gene expression and mutations are necessary elements in the process of carcinogenesis. Irrespective of the mechanisms by which these events may arise, oxidants are involved in each case.

Dietary antioxidants as inhibitors of oxidative damage to DNA and as a factor that decreases cancer risk

There is widely believed to be a link between diet and cancer incidence. A plethora of epidemiological studies have described the protective effects of diets rich in fruits and vegetables on some cancers [31, 70]. The mode of action of dietary micronutrients is complex and far from being fully understood. It is reasonable to assume that agents that decrease oxidative damage to DNA should also decrease risk of cancer development. One possible mechanism by which the protective effect of fruits and vegetables is exerted could thus be by way of the antioxidant activities of dietary components such as vitamins A, C and E or phenolic compounds. These antioxidants are effective free radical scavengers, and should protect DNA from oxidative damage. However, large-scale intervention studies have failed to demonstrate that intake of antioxidant vitamins decrease the cancer risk and meta-analysis even indicates increased risk of mortality [4].

Intuitively, supplementation trials would appear to represent the most relevant way of exploring antioxidant effects in humans, although the sampling most often is restricted to the use of surrogate tissues such as white blood cells (WBC) and urine. At the beginning of the 1990s, as the ability to detect 8-oxodG in DNA appeared, the first of many antioxidant supplementation trials dealing with this lesion in WBC was carried out [33]. Reliable detection of urinary 8-oxodG excretion became possible at the same time [41] which, coupled with the difficulties associated with artefactual formation of oxidative damage to DNA (see ESCODD, above), urinary 8-oxodG excretion became the favoured biomarker in antioxidant trials. However, by far the most popular assay in antioxidant intervention trials has been the comet assay with detection of DNA strand breaks (SB) or the enzyme-modified version of the comet assay that detects oxidized purines (including 8-oxodG) and pyrimidines by formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (ENDOIII), respectively. The literature on biomarkers of oxidative damage to DNA in WBC in a number of small-scale intervention studies of antioxidant supplements has been summarized in a series of reviews [45–47]. Many studies suffer, however, from non-optimum design. Table 1 provides an overview of intervention studies involving optimal design in which the effects of antioxidants or antioxidant-rich foods supplements on oxidative damage to DNA in WBC or in urine have been investigated.

Effect of antioxidant supplementation on oxidative damage to DNA in WBC

Ingestion of single doses of simple antioxidants is typically associated with temporarily reduced levels of oxidative damage to DNA [45–47]. The effect of a single dose of vitamin C seems to disappear within some hours, while tocopherols and carotenoids might exert their effects somewhat longer, possibly because of differences in the respective rates of bioavailability and elimination [5, 11, 14, 29, 36, 58]. Among the studies in which multiple doses of single antioxidants are given, there are fewer studies reporting protective effects than negative results [45–47]. This suggests that the protective effect of a single antioxidant is relatively short. In two well-designed studies of the effects of administrating a combination of antioxidant vitamins, a protective effect could be shown only after 20 weeks of supplementation but no effect after 10 weeks (or 6 months of supplementation) [17, 34]. Accordingly, the question of whether multiple vitamin supplementation provide better protection against oxidative damage to DNA than a single dose cannot be answered unambiguously.

Antioxidant rich foods

Ingestion of a diet rich in flavonols (including quercetin) and of one rich in cruciferous and legume sprouts (113 g/day for 2 weeks) was not found to alter the level of ENDOIII and FPG sites, respectively [24, 40]. Also, consumption of rye crisp bread (76.5 mg/day for 2 weeks), as a source of lignans, was neither associated with increased plasma enterolactone concentration, nor effect on ENDOIII sites [54]. The lack of effect of lignans in WBC would seem reasonable in the view of the low bioavailability of the active substances in rye crisp bread, and their effects in the

Table 1 Multiple administrations of dietary antioxidants with assessment of oxidatively DNA damage in white blood cells and urine

Supplement per day	Subjects[a]	Age (years)[b]	Effect	References
Rutin (500 mg) for 6 weeks	16 F (NR)	18–48	No effect on ENDOIII sites (Comet) in WBC or spot urinary excretion of 8-oxodG (HPLC)	[5]
α-carotene and β-carotene (15 mg) for 12 weeks	40 MF (NS)	25–45	No effect on ENDOIII and FPG sites (Comet) and 8-oxodG (HPLC) in WBC	[11]
β-carotene (20 mg) for 14 weeks	122 M (S)	39 (NR)	No effect on urinary excretion of 8-oxodG/24-h (HPLC)	[55]
β-carotene (30 mg) for 1 mo	14 M (NS)	19–22	No effect on urinary excretion of 8-oxodG/24-h (HPLC)	[62]
Carotenoids[c] for 3 weeks	32 MF (NS)	32 ± 11	No effect versus baseline, but decreased urinary excretion of 8-oxodG (ELISA) in active group post-supplementation	[38]
Vitamin C (500 mg) for 3 weeks	30 MF (NS)	17–49	No effect of 8-oxodG (ELISA) in spot urine due to vitamin C supplementation, but increased excretion in washout period	[12]
Vitamin C (80, 200, or 400 mg) for 15 weeks	154 MF (NS)	18–64	No effect on 8-oxodG (HPLC) in WBC	[29]
Vitamin E (1,000 IU) for 12 weeks prior to an exercise test (downhill run)	32 M (NS)	26 (3) and 71 (4)	Decreased 8-oxodG (HPLC) in WBC of the young subjects and no effect among the old	[58]
Vitamin E (800 mg) for 7 days prior to hyperbaric oxygn treatment	6 MF (NS)	20–39	No effect on ENDOIII and FPG sites (Comet) in WBC	[14]
Vitamin E (5–7 or 80 mg) together with diet containing either 5 or 15% polyunsaturated acids for 4 weeks	21 M (NS)	29 ± 1	Decreased ENDOIII sites (Comet) in WBC in the low vitamin E group	[36]
Six groups receiving combinations of vitamins[d] for 2 mo	116 M (S)	30–65	No effect of 24-h urinary excretion of 8-oxodG (HPLC)	[57]
Vitamin C (1 g) and vitamin E (0.6 g) for 1 mo	13 M (NR)	30 ± 3	Lower 24-h urinary excretion of 8-oxodG (HPLC) in the active group of HIV-infected patients receiving zidovudine therapy	[2]
2 × 2 parallel study of vitamin C (500 mg) and vitamin E (400 IU) for 2 mo	184 MF (NS)	58 ± 14	No effect on 24-h urinary excretion of 8-oxodG (ELISA)	[30]
2 × 2 parallel study of vitamin C (500 mg) and vitamin E 182 mg) for 12 mo	48 M (22S)	45–69	No effect on 24-h urinary excretion of 8-oxodG (HPLC)	[56]
Vitamin C (500 mg as plain or slow release formulations) and vitamin E (182 mg) for 4 weeks	48 M (S)	39 ± 12	Decreased ENDOIII and FPG sites (Comet) in the group that ingested tablets with the slow-release vitamin C formulation	[49]
Multi-vitamin tablet (100 mg vitamin C, 280 mg vitamin E, and 25 mg β-carotene) for 20 weeks	100 M (50S)	50–59	Decreased ENDOIII sites (Comet) after 20 weeks in WBC	[17]
Multi-vitamin (250 vitamin C, 200 IU α-tocopherol, and 6 mg β-carotene) for 6 mo	63 MF (S)	42 ± 9	No difference in 8-oxodG (antibody-based detection) in WBC between placebo and supplemented groups, but a decline in both groups during the trial	[34]
Multi-vitamin tablet[e] for 14 days	30 NR (NR)	22 ± 1	No effect of 24-h urinary excretion of 8-oxodG in subjects undergoing cold-weather training at moderate altitude	[53]
Multi-vitamin tablet[f] for 21 days	39 MF (NR)	7 ± 2	No effect on excretion of 8-oxodG (ELISA) in spot urine samples	[60]
Multi-vitamin tablet[g] for 24 days	40 M (NR)	18–40	No effect on overnight excretion of 8-oxodG (ELISA) in subjects undergoing cold-weather training at moderate altitude	[59]
Multi-vitamin tablet[h] for 35 days	18 M (NS)	26 ± 5	No effect on urinary excretion of 8-oxodG/24-h (HPLC) among subjects exposed to high altitude hypoxia	[61]
Fruit and vegetable capsules[i] for 7 weeks	59 MF (11S)	50 ± 6	No effect on excretion of 8-oxodG (ELISA) in spot urine samples	[37]
Antioxidant rich diet or tablets[j] for 5 weeks	55 MF (NR)	71 ± 6	No effect versus baseline, but decreased 24-h urinary excretion of 8-oxodG (ELISA) in active group post-supplementation	[51]
Rye crisp bread (76.5 g) or placebo (fibre-free bread) for 2 weeks	12 F (NR)	NR	No effect on ENDOIII sites (Comet) in WBC	[54]
Prebiotic bread with and without antioxidants (including green tea, spices and tomato) for 5 weeks	38 MF (20S)	20–55	No effect on ENDOIII sites (Comet) in WBC	[25]
Flavonol (quercetin) rich diet for 2 weeks in crossover design among type 2 diabetes patients	10 MF (3S)	60 ± 7	No effect on ENDOIII sites (Comet) in WBC	[40]
Vegetable/fruit (500 g) in crossover design for 3 weeks with 2 weeks washout	22 M (S)	33 ± 11	No effect on ENDOIII sites (Comet) in WBC	[3]
Vegetable/fruit (600 g) or tablets with the same concentration of antioxidants/minerals for 24 days	43 MF (NS)	27 ± 6	No effect on ENDOIII and FPG sites (Comet) in WBC. No effect on 24-h urinary excretion of 8-oxodG (HPLC), but a decline in all groups	[50]
Vegetable/fruit (516 or 1,059 g/10 M) and different intake of fatty acids (3 or 11% of the energy) for 6 weeks	96 MF (S)	19–52	No effect on the urinary excretion of 8-oxodG/48-h	[21]

Table 1 Continued

Supplement per day	Subjects[a]	Age (years)[b]	Effect	References
Vegetable consumption (3.6 vs. 12 servings) for 2 weeks	64 F (NR)	23–81	Lower 8-oxodG in WBC (HPLC) among those subjects eating 12 servings per day	[63]
Cruciferous and legume sprouts (113 g) for 2 weeks	18 MF (NR)	21–45	No effect on FPG sites (Comet) in WBC	[24]
Plant extract of *Rosa roxburghii* for 3 weeks	33 MF (NS)	21–27	No effect on the urinary excretion of 8-oxodG in spot urine samples (ELISA)	[35]
Kiwi fruit (1–3 pieces) for 3 weeks	14 MF (NS)	26–54	Decreased ENDOIII and FPG (Comet) sites	[10]
Brussels sprouts (300 g) for 1 weeks	10 MF (NS)	NR	No effect on 24-h urinary excretion of 8-oxodG (HPLC)	[67]
Brussels sprouts (300 g) for 12 days	10 M (NS)	NR	Decreased 24-h urinary excretion of 8-oxodG (HPLC) in the active group	[66]
Fruit juice (480 ml)[k] for 4 days	11 M (NR)	21 ± 1	Decreased 12-h urinary excretion of 8-oxodG (ELISA)	[20]
Cranberry juice (750 ml) for 2 weeks	20 F (2S)	18–40	No effect on ENDOIII (Comet) in WBC	[16]
Blackcurrant juice or anthocyanine drink (475–1,000 ml) for 3 weeks	57 MF (6S)	19–52	No effect on ENDOIII and FPG sites (Comet) in WBC	[48]
Green tea or black tea (four cups) for 1–4 mo	120 MF (S)	18–79	Decreased excretion of 8-oxodG (ELISA) in spot urine samples after 4 mo in green tea group, but not before	[26, 27]
Two interventions of green tea with 300 ml for 7 days or 32 oz for 7 days	68 MF (13S)	18–45	Decreased 12-h urinary excretion of 8-oxodG (HPLC)	[39]
Green tea extract[l] for 3 weeks	16 M (8S)	20–31	No effect related to supplementation on 24-h urinary excretion of 8-oxodG (HPLC) but a decreased excretion during the study in all groups	[71]
Green tea (500 or 1,000 mg) among high-risk subjects of liver cancer for 3 months	124 MF (NR)	NR	Lower 8-oxodG/24-h (HPLC) after 3 months supplementation but not after 1 month	[42]
Soya-hypocotyl tea (>1,000 ml) for 1 mo	38 F (NR)	NR	Decreased excretion of 8-oxodG (ELISA) in active group (statistical test not reported)	[68]
Soy milk, rice milk, or cow milk (1 l) for 4 weeks	10 M (NS)	20–50	Decreased ENDOIII sites (Comet) for soy milk	[44]
Polyphenol-rich olive oils (25 ml) for 40 days	12 M (NS)	20–22	Decreased excretion of 8-oxodG (HPLC) in spot urinary samples following supplementation in a dose-dependent manner	[69]
Olive oil (25 ml) with three different content of phenolic compounds for 3 weeks	182 M (NS)	20–60	Decreased 8-oxodG/24-h urinary excretion, but no difference in urinary excretion of 8-oxoGua/24-h. No difference in the effect of the different olive oils	[43]

[a]Number of subjects indicated as males (*M*) and females (*F*). Smokers (*S*) and non-smokers (*NS*) are indicated in brackets. Lacking information is indicated as NR (not reported)
[b]Age is shown as range or mean ± standard deviation
[c]Supplement constitute β-carotene (6 mg), α-carotene (1.4 mg), lycopene (4.5 mg), bixin (11.7 mg), lutein (4.4 mg), and paprika carotenoids (2.2 mg)
[d]The six groups had daily supplementations with (1) 200 mg vitamin E; (2) 500 mg plain-release vitamin C; (3) 500 mg slow-release vitamin C; (4) 90 mg coenzyme Q_{10} in oil; (5) 30 mg coenzyme Q_{10} granulate; (6) placebo
[e]Tablets contain β-carotene (12 mg), vitamin E (400 IU), vitamin C (500 mg), selenium (100 μg), and zinc (30 mg)
[f]Tablets contain micronutrients similar to three fruits and three vegetables (including 107 mg vitamin C and 83 mg vitamin E)
[g]Consisted of β-carotene (20,050 IU), vitamin C (330 mg), tocopherols (650 IU), selenium (167 μg), catechin (13.2 mg), lutein (500 μg), lycopene (100 μg), *N*-acetyl-1-cysteine (181 mg), and pomegranate extract (5 mg)
[h]Consisted of β-CT (20,000 IU), α-tocopherol (400 IU), vitamin C (500 mg), selenium (100 μg), and zinc (30 mg)
[i]The fruit capsules were made from juiced apple, orange, pineapple, papaya, cranberry and peach, and the vegetable capsules were from carrots, parsley, beet, broccoli, kale, cabbage, spinach, and tomato. The daily dose consisted of 200 mg vitamin C, 60 mg vitamin E, and 15 mg β-carotene
[j]Consisted of tablets with vitamin antioxidants (400 mg vitamin C, 150 mg vitamin E, 4 mg β-carotene), capsules (90 mg vitamin C, 18 IU vitamin E, 2.4 mg β-carotene, and powder or extract of fruits and berries), or a carotenoid-rich diet
[k]Contain vitamin A (240 mg), β-carotene (399 μg), vitamin C (219 mg), vitamin E (1.44 mg) per day.
[l]Consisted of 1,000 mg extract/kg bodyweight in meat patties (total phenolics were 23.5 mg/10 MJ)

gastrointestinal tract are easier to comprehend. Drinking blackcurrant juice or an anthocyanine drink (475–1,000 ml/day for 3 weeks) also had no beneficial effect on ENDOIII and FPG sites. In fact, there was a tendency that the level of FPG sites increased in the group of subjects drinking blackcurrant juice [48]. Anthocyanines have low bioavailability and the dose provided here was rather high. It may be speculated that the subjects had a slight, unintentional, intoxication of the gastrointestinal tract (some of the subjects in the active groups complained of nausea, for example). Three studies have investigated the effects of supplementation of vegetables and fruits. The one investigation, a crossover study with male smokers, showed no effect of ENDOIII sites of ingesting 500 g/day of such food for 3 weeks [3]. The other, a placebo-controlled parallel study in which non-smoking subjects of both sexes were given 600 g/day of fruits and vegetables for 24 days, was negative with respect to effects on the ENDOIII and FPG sites [50]. The third study supplemented with 12 servings per day of vegetables and reported a modest (16.5%) lower level of 8-oxodG as compared to the reference group that ingested 3.6 servings per day [63]. Five studies carried out, in which a reduction in the level of oxidative damage to DNA was observed, provided very different antioxidant-rich foods that are not easy to compare. Drinking soy milk (1,000 ml/day for 4 weeks) as a source of phytoestrogens in one study increased plasma levels of genistein and daidzein but not enterolactone; assessment of DNA damage revealed lower levels of ENDOIII [44]. The only study showing consistent effects of more than one endpoint was a study of effect of kiwi fruit supplementation (1–3 kiwi fruits/day for 3 weeks), which showed the numbers of ENDOIII and FPG sites to be reduced [10].

An overall summary of the studies showed that seven investigations reported beneficial effect of antioxidant supplementation [10, 17, 36, 44, 49, 58, 63], whereas 13 studies reported null effect [3, 5, 11, 14, 16, 24, 25, 29, 34, 40, 48, 50, 54]. There is little support for the notion that ingestion of antioxidant-rich foods is associated with lower spontaneous level of oxidative damage to DNA in WBC than intake of single antioxidants.

Effect of antioxidant supplementation on 8-oxodG in urine

Measurement of urinary excretion of 8-oxodG in antioxidant intervention studies is based on the notion that it decreases following a steady state ingestion of antioxidants because of the rate of generation of oxidative damage to DNA in the body being decreased. For 28 studies of the effect of antioxidant supplementation on urinary excretion of 8-oxodG, in which controlled design was employed [2, 5, 12, 20, 21, 27, 30, 35, 37–39, 42, 43, 50, 51, 53, 55–57, 59–62, 66–69, 71], no appreciable difference was present in terms of duration of the intervention period, number of subjects, or the power to detect a 50% change between studies reporting beneficial effects and those reporting no effects. Overall there were 11 investigations showing beneficial effect of antioxidant supplementation [2, 20, 26, 38, 39, 42, 43, 51, 66, 68, 69], whereas 17 studies reported that the supplementation had no effect on the urinary excretion of 8-oxodG, during the period of supplementation [5, 12, 21, 30, 35, 37, 50, 53, 55–57, 59–62, 67, 71].

Four studies on single carotenoid supplementation showed no effect on urinary excretion of 8-oxodG [5, 55, 57, 62]. A comparable study involving supplementation of a mixture of carotenoids revealed a statistically significant difference in delta values (i.e. the difference between data obtained at the end of the supplementation and baseline) between the active and placebo groups, but there was no difference relative to the baseline values [38]. In fact, the statistically significant difference of the delta values was mainly caused by an increased 8-oxodG excretion in the placebo group, whereas the excretion only decreased slightly in the group receiving mixed carotenoids. In four different studies, supplementation with single doses of or a combination of vitamin C and vitamin E had no effect in healthy subjects [12, 30, 56, 57], whereas one study reported a beneficial effect of high dose supplementation with vitamin C (1,000 mg/days) and vitamin E (600 mg/days) in HIV-infected patients that had received a drug (zidovudine) that causes oxidation of DNA in both mice and humans [2]. Studies on multi-vitamin tablet supplementation have shown no beneficial effect in neither normal subjects [50, 51, 60] nor in subjects exposed to altitude hypoxia with or without cold-weather field training [53, 59, 61].

Investigations of natural food products show an equal distribution between the studies reporting beneficial and null effect. Ingestion of olive oils with high content of phenolic compounds was associated with lower urinary excretion of 8-oxodG [69]. Another study also showed lower level of urinary 8-oxodG excretion following supplementation with three different olive oils, but the effect was not related to the content of phenolic compounds in the olive oils [43]. Interestingly, this study also included measurements of the urinary excretion of 8-oxoGua, which is considered to be the primary degradation product of the Ogg1-mediated repair of 8-oxodG in genome, but the supplementation did not affect the 8-oxoGua excretion [43]. A number of studies have involved

supplementation of berries, fruits, tea, and vegetables. Taking capsules containing extracts of fruits and berries, and eating diets rich in carotenoids were both found to lower the excretion of 8-oxodG [51]. A beneficial effect of vegetable juice consumption on 8-oxodG excretion was also observed in subjects enrolled in a soccer summer training camp [20]. On the other hand, neither eating 600 g of fruit and vegetable nor the corresponding amount of minerals and vitamins in tablet form were associated with lower urinary excretion of 8-oxodG relative to the placebo group, whereas there was a pronounced period effect of the intervention [50]. Another study of parallel groups receiving varying content of polyunsaturated fatty acids and fruits and vegetables showed that the urinary excretion of 8-oxodG tended to decrease in all groups, whereas there was no difference in the effect between the groups [21]. Ingestion of capsules containing juices and powder of fruits and vegetables had no effect of 8-oxodG excretion in urine [37].

Mixed results concerning the effect of a dietary supplementation of Brussels sprouts (300 g/day) were obtained in two studies [66, 67]. In the first study, which involved only male subjects, the Brussels sprouts supplementation lowered urinary excretion of 8-oxodG [66]. In the subsequent study, in which both sexes were included, the effect was less clear, there being a tendency that only the males to benefit from ingestion of Brussels sprouts, but the results were not firm because of the small number of subjects tested and of one of the male subjects showing an unexpectedly high urinary 8-oxodG excretion level [67]. Four other studies in this area concerned the effect of drinking green tea on ingestion of green tea extracts [26, 27, 39, 42, 71]. In one study, there was a beneficial effect of drinking 300 ml/day for a week [39], whereas there was no effect of ingesting green tea extract in meat patties for 3 weeks [71]. The unadjusted data of the third study indicated no beneficial effect of drinking green tea, but adjustment of the data for number of variables such as baseline 8-oxodG levels, revealed a statistically significant effect of drinking green for 4 months [26, 27]. Drinking green tea also lowered the urinary excretion of 8-oxodG in subjects who were at high-risk of developing liver cancer [42]. In still a further study, drinking soya hypocotyl tea was associated with lower urinary excretion of 8-oxodG, although it should be emphasized that the fate of the antioxidants in this investigations was inconclusive since (1) the plasma concentration of carotenoids decreased; (2) the putative active constituents (isoflavones) were not measured in the plasma, and (3) the alterations in the urinary concentration could not be assessed due to insufficient information [68]. Drinking extracts from sweet chestnut rose (*Rosa roxburghii*) plant or cranberry juice did not altered 8-oxodG excretion [16, 35].

There are a number of reasons why a positive effect of the vitamin supplementation on cancer risk, in connection with oxidative damage to DNA, might not have been observed;

1. It is possible that a protective effect from vitamin supplementation may only be seen when their basal levels are very low such as in the case of severe oxidative stress. Indeed, vitamin supplementation of HIV-infected patients, who had low levels of antioxidant vitamins and high 8-oxodG levels in lymphocyte, resulted in the restoration of the vitamin levels characteristic for the control subjects. Concomitantly, the authors also noted a significant decrease in the levels of the modified bases, compared to the patients who received placebo [2]. It is possible, therefore, that the presence of oxidative stress, which might fail to be recognized, could increase the likelihood of detecting a protective effect.
2. Under some of circumstances, antioxidant vitamins (vitamin C and A) might exert pro-oxidative effects. It is possible that supplementation of vitamin C in subjects with iron-overload may increase the level of oxidative damage to DNA. It is worthy of note in this context that presumably healthy subjects might have the hereditary disease idiopathic haemachromathosis, which leads to iron overload, such that iron catalytic for free radical reactions (the so-called labile iron pool) is increased in plasma [28]. Interestingly, a positive correlation has been demonstrated between the level of the labile iron pool and the level of 8-oxodG in lymphocytes from putatively healthy subjects as well a population of humans characterized by having mild hypercholesterolemia [22, 65]. Moreover, the absorption of non-haem iron has also been found to be affected by ascorbic acid [23].
3. It can be speculated whether the concentration of antioxidants in blood and 8-oxodG in lymphocytes or leukocytes DNA are representative measures of the situation in the target tissue of the carcinogenesis.
4. It is possible that the antioxidants themselves may allow clonal expansion and tumour promotion by protecting initiated cells from oxidant toxicity and apoptosis that would otherwise kill them [7].
5. Antioxidant vitamins may have other biological activities that are not related to the effect of inhibiting oxidizing reactions. For instance, antioxidant vitamins appear to be able to affect the regulation of gene expression [1].

Comments

There are numerous experimental studies published each year on the potential of antioxidants or antioxidant-rich foods to prevent the oxidation of DNA. On the experimental basis, it is easy to understand the ingestion of antioxidants being associated with lower levels of oxidative damage to DNA. However, many of the studies are of only limited value because of methodological problems related to the study design and the assays, and many lack the power to detect 50% differences between two study groups. Although single studies may have higher power due to specific designs such as repeated measurements, it is a major concern that many studies have too few subjects. The effect-ratio in healthy subjects is most likely less than 10%, which means that the number of subjects should be in the hundreds rather than tens [47].

At present, no firm conclusions can be reached on the basis of antioxidant intervention studies. There is a tendency that supplementation with antioxidant-rich food decreases the urinary excretion of 8-oxodG, whereas single antioxidants do not. Studies of the effect in WBC indicate little support for the notion that long-term antioxidant supplementation lowers the basal level of oxidative damage to DNA, although there may be a beneficial effect in the first few hours after ingestion of vitamin antioxidant or phytochemicals. This can interpreted as antioxidants having an overall beneficial effect on the body as a whole, yet the use of WBC as surrogate tissue may not be particularly well suited for detection of this effect. It should also be borne in mind that the majority of studies involve healthy individuals, whereas the protective effect of antioxidants might be easier to detect in subjects who suffer from oxidative stress. These could be either healthy subjects exposed to oxidative stress (such as exhaustive exercise and hyperbaric oxygen treatment) or patients under oxidative stress as a result of existing disease. However, the few well-controlled studies that have reported realistic levels of oxidative damage to DNA in WBC of oxidatively stressed subjects lend little support to the notion that such a population benefits more from antioxidant supplementation than a population of normal subjects. It should be noted, though, that most of the investigations have used vitamin E supplementation, which is anticipated to be the least effective of antioxidants with which to supplement. There is an obvious need for controlled antioxidant intervention studies encompassing subjects who are oxidatively stressed and in whom oxidative damage to DNA is measured by enzymic or chromatographic methods, and also urinary assessments of 8-oxodG.

In the future, more attention should probably be devoted to alternative chemopreventive mechanisms such as the upregulation of DNA repair systems and to other types of DNA damage, such as bulky DNA adducts. Also the chemopreventive effect of antioxidants in non-lymphatic tissue, which has only been sparsely investigated, should be addressed thoroughly before the idea of antioxidants having clearly beneficial effects is abandoned. Hopefully, such studies will benefit from the lessons learned from antioxidant intervention studies, particularly as regards for the need of proper investigative designs and of the validity of the biomarkers, which are of pivotal importance for such studies.

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Conflict of interest none.

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